Application of molecular sexing in the Great Hornbill (*Buceros bicornis*) toward captive breeding management at the Zoological Parks Organization of Thailand.

Janjira Sermsiri[2], Yollada Taengphukhieo[1], Supranee Chanmueangthai[1], Ampa Hothaisong[1], Worawidh Wajjwalku[3] , Jiranan Insee[4] and Chainarong Punkong[2]

[1] Conservation, Research and Animal health Department, Khao Kheow Open Zoo, Chonburi Province, Thailand.
[2] Plant Genetic Conservation Project Under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG).
[3] Wildlife Clinic, Kasetsart University Veterinary Teaching Hospital, Kamphaengsaen, Nakhonpathom Province, Thailand.
[4] Faculty of Agro-Industrial Technology, Kalasin University, Kalasin Thailand.

Thailand is a stronghold of 13 species of hornbill. Effective management of both captive and wild populations plays an important role in species survival. In the Great Hornbill (*Buceros bicornis*) molecular sexing overcomes the limitation in subtle morphological differences and enables rapid, accurate sex identification in this monomorphic species. In this study, we developed four sets of sexing primers and protocol to provide information on sex and Z-chromosome diversity in captive populations (N=18) in Thailand using intron length polymorphism and Single Nucleotide Polymorphism (SNP) patterns of Chromo-Helicase DNA (CHD) gene (CHD-Z and CHD-W). Based on the primer set of 2550/2718, 2551/2718 and 2561/2728, individuals identified as male (ZZ) had a single band at 600bp (CHD-Z), while the female (ZW) had two bands with the sizes of 400bp (CHD-W) and 600bp (CHD-Z). The system of primer P2/P8, followed by Restriction Length Polymorphism (RFLP), was able to accurately identify sex with the presence of a single band (600bp) as 642bp and two bands (400bp) as 401bp. In addition to sex identification, genetic diversity assessment based on SNP in CHD-Z remains limited. After sequencing of the PCR products of CHD-Z, we detect SNP sites and provide insight into CHD-Z diversity useful for screening and monitoring genetic variation and adaptive potential of founders.

Keywords : molecular sexing, Hornbill, CHD-Z gene, nucleotide sequence

Corresponding author : Chainarong Punkong (agist_C@hotmail.com)